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(54) Title: A NOVEL METHOD OF DIAGNOSING, MONITORING, AND STAGING COLON CANCER

(57) Abstract

The present invention provides a new method for detecting, diagnosing, monitoring, staging, and prognosticating colon cancer.

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A NOVEL METHOD OF DIAGNOSING, MONITORING, AND STAGING COLON CANCER

FIELD OF THE INVENTION

This invention relates, in part, to newly developed assays for detecting, diagnosing, monitoring, staging, and prognosticating cancers, particularly colon cancer.

BACKGROUND OF THE INVENTION

Colon cancer is the second most frequently diagnosed malignancy in the United States. Cancer of the gastrointestinal tract, especially colon cancer, is a highly treatable and often a curable disease when localized to the bowel. However, currently colon cancer is the second most common cause of cancer death. Surgery is the primary treatment and results in cure in approximately 50% of patients. Recurrence following surgery is a major problem and often is the ultimate cause of death. The prognosis of colon cancer is clearly related to the degree of penetration of the tumor through the bowel wall and the presence or absence of nodal involvement. These two characteristics form the basis for all staging systems developed for this disease. Bowel obstruction and bowel perforation are indicators of poor prognosis. Elevated pretreatment serum levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) also have negative prognostic significance.

Because of the frequency of the disease (approximately 160,000 new cases of colon cancer per year), the identification of high-risk groups, the demonstrated slow growth of primary lesions, the better survival of early-stage lesions, and the relative simplicity and accuracy of screening tests, screening for colon cancer should be a part of routine

care for all adults starting at age 50, especially those with first-degree relatives with colorectal cancer.

Procedures used for detecting, diagnosing, monitoring, staging, and prognosticating colon cancer are of critical importance to the outcome of the patient. For example, patients diagnosed with early colon cancer generally have a much greater five-year survival rate as compared to the survival rate for patients diagnosed with distant metastasized colon cancer. Treatment decisions are usually made in reference to the older Dukes or the Modified Astler-Coller (MAC) classification schema for staging. However, new diagnostic methods which are more sensitive and specific for detecting early colon cancer are clearly needed.

Further, colon cancer patients must be closely monitored following initial therapy and during adjuvant therapy to determine response to therapy and to detect persistent or recurrent disease of metastasis. Thus, there is clearly a need for a colon cancer marker which is more sensitive and specific in detecting colon cancer recurrence.

Another important step in managing colon cancer is to determine the stage of the patient's disease. Stage determination has potential prognostic value and provides criteria for designing optimal therapy. Currently, pathological staging of colon cancer is preferable over clinical staging as pathological staging provides a more accurate prognosis. However, clinical staging would be preferred were the method of clinical staging at least as accurate as pathological staging because it does not depend on an invasive procedure to obtain tissue for pathological evaluation. Staging of colon cancer would be improved by detecting new markers in cells, tissues, or bodily fluids which could differentiate between different stages of invasion.

In the present invention, methods are provided for detecting, diagnosing, monitoring, staging, and

prognosticating colon cancers, particularly colon, stomach, and small intestine cancer, via nine (9) Colon Specific Genes (CSGs). The nine CSGs refer, among other things, to native proteins expressed by the genes comprising the polynucleotide sequences of any of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8 or 9. In the alternative, what is meant by the nine CSGs as used herein, means the native mRNAs encoded by the genes comprising any of the polynucleotide sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8 or 9 or levels of the genes comprising any of the polynucleotide sequences of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8 or 9.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide a method for diagnosing the presence of colon cancer in a patient which comprises measuring levels of CSG in a sample of cells, tissue or bodily fluid from the patient and comparing the measured levels of CSG with levels of CSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in the measured CSG levels in the patient versus levels of CSG in the control is associated with colon cancer.

Another object of the present invention is to provide a method of diagnosing metastatic colon cancer in a patient which comprises measuring CSG levels in a sample of cells,

tissue, or bodily fluid from the patient and comparing the measured CSG levels with levels of CSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured CSG levels in the patient versus levels of CSG in the control is associated with a cancer which has metastasized.

Another object of the present invention is to provide a method of staging colon cancer in a patient which comprises identifying a patient having colon cancer, measuring levels of CSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured CSG levels with levels of CSG in preferably the same cells, tissue or bodily fluid type of a control. An increase in measured CSG levels in the patient versus CSG levels in the control can be associated with a cancer which is progressing while a decrease or equivalent level of CSG measured in the patient versus the control can be associated with a cancer which is regressing or in remission.

Another object of the present invention is to provide a method of monitoring colon cancer in a patient for the onset of metastasis. The method comprises identifying a patient having colon cancer that is not known to have metastasized, periodically measuring levels of CSG in a sample of cells, tissues, or bodily fluid obtained from the patient, and comparing the measured CSG levels with levels of CSG in preferably the same cells, tissue, or bodily fluid type of a control, wherein an increase in measured CSG levels versus control CSG levels is associated with a cancer which has metastasized.

Yet another object of the present invention is to provide a method of monitoring the change in stage of colon cancer in a patient which comprises identifying a patient having colon cancer, periodically measuring levels of CSG in a sample of cells, tissue, or bodily fluid obtained from the patient, and comparing the measured CSG levels with levels of

CSG in preferably the same cells, tissues, or bodily fluid type of a control wherein an increase in measured CSG levels versus the control CSG levels is associated with a cancer which is progressing and a decrease in the measured CSG levels versus the control CSG levels is associated with a cancer which is regressing or in remission.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging, and prognosticating cancers by comparing levels of CSG with those of CSG in a normal human control. What is meant by "levels of CSG" as used herein, means levels of the native protein expressed by the genes comprising the polynucleotide sequence of any of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9. In the alternative, what is meant by "levels of CSG" as used herein, means levels of the native mRNA encoded by any of the genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9 or levels of the gene comprising any of the polynucleotide sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9. Such levels are preferably measured in at least one of, cells, tissues and/or bodily fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for

diagnosing over-expression of any one of the CSG proteins compared to normal control bodily fluids, cells, or tissue samples may be used to diagnose the presence of cancers, including colon cancer. Any of the nine CSGs may be measured alone in the methods of the invention, or all together or any combination of the nine.

By "control" it is meant a human patient without cancer and/or non cancerous samples from the patient, also referred to herein as a normal human control. in the methods for diagnosing or monitoring for metastasis, control may also include samples from a human patient that is determined by reliable methods to have colon cancer which has not metastasized.

All the methods of the present invention may optionally include measuring the levels of other cancer markers as well as CSG. Other cancer markers, in addition to CSG, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art.

Diagnostic Assays

The present invention provides methods for diagnosing the presence of colon cancer by analyzing for changes in levels of CSG in cells, tissues or bodily fluids compared with levels of CSG in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase in levels of CSG in the patient versus the normal human control is associated with the presence of colon cancer. Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the patient being tested has cancer is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal human control.

The present invention also provides a method of diagnosing metastatic colon cancer in a patient having colon

cancer which has not yet metastasized for the onset of metastasis. In the method of the present invention, a human cancer patient suspected of having colon cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a variety of means known to those of skill in the art. For example, in the case of colon cancer, patients are typically diagnosed with colon cancer following traditional detection methods.

In the present invention, determining the presence of CSG level in cells, tissues, or bodily fluid, is particularly useful for discriminating between colon cancer which has not metastasized and colon cancer which has metastasized. Existing techniques have difficulty discriminating between colon cancer which has metastasized and colon cancer which has not metastasized and proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker levels measured in such cells, tissues, or bodily fluid is CSG, and are compared with levels of CSG in preferably the same cells, tissue, or bodily fluid type of a normal human control. That is, if the cancer marker being observed is just CSG in serum, this level is preferably compared with the level of CSG in serum of a normal human patient. An increase in the CSG in the patient versus the normal human control is associated with colon cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferable are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal patient.

Normal human control as used herein includes a human patient without cancer and/or non cancerous samples from the patient; in the methods for diagnosing or monitoring for metastasis, normal human control may also include samples from a human patient that is determined by reliable methods to have colon cancer which has not metastasized.

Staging

The invention also provides a method of staging colon cancer in a human patient.

The method comprises identifying a human patient having such cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG. Then, the method compares CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Monitoring

Further provided is a method of monitoring colon cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Further provided by this inventions is a method of monitoring the change in stage of colon cancer in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample

of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease in the levels of CSG is associated with a cancer which is regressing in stage or in remission.

Monitoring such patient for onset of metastasis is periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the particular patient, and the stage of the cancer.

Assay Techniques

Assay techniques that can be used to determine levels of gene expression, such as CSG of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses and ELISA assays. Among these, ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids. An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to CSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to CSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, for example horseradish peroxidase enzyme or alkaline phosphatase.

To carry out the ELISA, antibody specific to CSG is incubated on a solid support, e.g., a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific

protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time CSG binds to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to CSG and linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to CSG. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to CSG antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to the amount of CSG protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to CSG attached to a solid support and labeled CSG and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of CSG in the sample. Nucleic acid methods may be used to detect CSG mRNA as a marker for colon cancer. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR

can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e., gridding) can be used to both detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the CSG gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding the CSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA, isolated from the tissue of interest. Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including but not limited to radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of gene expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and then using that material to generate a standard curve.

The above tests can be carried out on samples derived from a variety of patients' cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) such as from tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva, or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum, or any derivative of blood.

EXAMPLES

The present invention is further described by the following examples. These examples are provided solely to illustrate the invention by reference to specific embodiments.

These exemplifications, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

Example 1: CSGs

Searches were carried out and CSGs identified using the following Search Tools as part of the LIFESEQ® database available from Incyte Pharmaceuticals, Palo Alto, CA:

1. Library Comparison (compares one library to one other library) allows the identification of clones expressed in tumor and absent or expressed at a lower level in normal tissue.

2. Subsetting is similar to library comparison but allows the identification of clones expressed in a pool of libraries and absent or expressed at a lower level in a second pool of libraries.

3. Transcript Imaging lists all of the clones in a single library or a pool of libraries based on abundance. Individual clones can then be examined using Electronic Northerns to determine the tissue sources of their component ESTs.

4. Protein Function: Incyte has identified subsets of ESTs with a potential protein function based on homologies to known proteins. Some examples in this database include Transcription Factors and Proteases. We identified some leads by searching in this database for clones whose component ESTs showed disease specificity.

Electronic subtractions, transcript imaging and protein function searches were used to identify clones, whose component ESTs were exclusively or more frequently found in libraries from specific tumors. Individual candidate clones were examined in detail by checking where each EST originated.

Table 1: CSGs

SEQ ID NO:	Clone ID #	Gene ID #	
1	238330	242807	Transcript Imaging
2	1285234	239588	Subsetting
3	1341701	29634	Transcript Imaging
4	816257	233421	Subsetting
5	775133	245080	Subsetting
6	1335450	245811	Subsetting
7	2348122	233711	Transcript Imaging
8	3228674	230273	Subsetting
9	1632174	229022	Transcript Imaging

The following example was carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following example can be carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Example 2: Relative Quantitation of CSG Gene Expression

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample are used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

To evaluate the tissue distribution, and the level of CSGs in normal and tumor tissue, total RNA was extracted from normal tissues, tumor tissues, and from tumors and the corresponding matched normal tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Taqman probe specific to the CSG. The results were analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the CSG compared to the calibrator.

Comparative Examples

Similar mRNA expression analysis for genes coding for the diagnostic markers PSA (Prostate Specific Antigen) and PLA2 (Phospholipase A2) was performed for comparison. PSA is currently the only cancer screening marker available in clinical laboratories. When the panel of normal pooled tissues was analyzed, PSA was expressed at very high levels in prostate, with a very low expression in breast and testis. After analysis of more than 55 matching samples from 14 different tissues, the data corroborated the tissue specificity seen with normal tissue samples. PSA expression was compared in cancer and normal adjacent tissue for 12 matching samples of prostate tissue. The relative levels of PSA were higher in 10 cancer samples (83%). Clinical data

recently obtained support the utilization of PLA2 as a staging marker for late stages of prostate cancer. mRNA expression data described herein showed overexpression of the mRNA in 8 out of the 12 prostate matching samples analyzed (66%). PLA2 had high levels of mRNA expression in small intestine, prostate, liver, and pancreas.

Measurement of SEQ ID NO:3; Clone ID 1341701; Gene ID 29634 (Cln106)

Absolute numbers are depicted in Table 2 as relative levels of expression of Cln106 (SEQ ID NO:3) in 12 normal different tissues. All the values are compared to normal testis (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 2: Relative levels of Cln106 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	110
Endometrium	0
Kidney	0
Liver	0
Ovary	0
Pancreas	0
Prostate	16
Small Intestine	0
Spleen	0
Stomach	0
Testis	1
Uterus	0

The relative levels of expression in Table 2 show for the CSG Cln106 (SEQ ID NO:3), mRNA expression is more than 6 fold higher in the pool of normal ascending colon (110) compared with prostate (16). Testis, the calibrator, with a relative expression level of 1, is the only other tissue expressing the mRNA for Cln106 (SEQ ID NO:3). These results demonstrate that mRNA expression of this CSG is highly specific for colon.

The absolute numbers in Table 2 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 3.

The absolute numbers in Table 3 are relative levels of expression of Cln106 (SEQ ID NO:3) in 57 pairs of matching samples. All the values are compared to normal testis (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 3: Relative levels of Cln106 Expression in Individual Samples

Sample ID	Tissue	Cancer	Matching Normal Adjacent
Sto AC93	Stomach 1	4	96
Sto AC99	Stomach 2	0.4	0.5
Sml 21XA	Small Intestine 1	0	0
Sml H89	Small Intestine 2	0.93	1.28
Cln B56	Colon-Cecum(A)1	317	101
Cln AS45	Colon-Ascending(A)2	316.3	146.5
Cln CM67	Colon-Cecum(B)3	481.0	217.5
Cln AS67	Colon-Ascending(B)4	858.1	220.6
Cln AS43	Colon-Ascending(C)5	1370	98
Cln AS46	Colon-Ascending(C)6	3051	375
Cln AS98	Colon-Ascending(C)7	26	42
Cln AS89	Colon-Ascending(D)8	524.6	11.0
Cln TX01	Colon-Transverse(B)9	2886.3	1992.0
Cln TX89	Colon-Transverse(B)10	146.0	35.9
Cln TX67	Colon-Transverse(C)11	2.9	421.7
Cln MT38	Colon-Splenic Flexture(M)12	1681	187

Cln SG89	Colon-Sigmoid(B)13	1063.8	31.1
Cln SG67	Colon-Sigmoid(C)14	8.5	9.4
Cln SG33	Colon-Sigmoid(C)15	264	549
Cln SG45	Colon-Sigmoid(D)16	580.0	114.6
Cln B34	Colon-Rectosigmoid(A)17	97	244
Cln CXGA	Colon-Rectum(A)18	45.1	273.4
Cln RC67	Colon-Rectum(B)19	2.7	20.0
Cln C9XR	Colon-Rectosigmoid(C)20	609	460
Cln RS45	Colon-Rectosigmoid(C)21	472.8	144.0
Cln RC01	Colon-Rectum(C)22	568	129
Cln RC89	Colon-Rectum(D)23	4.6	322.91
Bld 46XK	Bladder 1	0.2	0
Bld 66X	Bladder 2	1	1
Bld 32XK	Bladder 3	0.0	0.0
Kid 126XD	Kidney 1	0	0
Kid 12XD	Kidney 2	0	0
Kid 5XD	Kidney 3	0.0	1.0
Kid 6XD	Kidney 4	0.0	0.0
Kid 106XD	Kidney 5	0.4	0.0
Liv 42X	Liver 1	0.0	0.0
Liv 15XA	Liver 2	0.0	0.0
Liv 94XA	Liver 3	0.0	0.0
Lng AC69	Lung 1	2	0
Lng BR94	Lung 2	0	0
Lng 47XQ	Lung 3	0	0
Mam 59X	Mammary Gland 1	0	0
Mam B011X	Mammary Gland 2	0	0
Mam A06X	Mammary Gland 3	0	0
Ovr 103X	Ovary 1	0.04	2.08
Ovr 130X	Ovary 2	0.1	2.76

Pan 71XL	Pancreas 1	4.08	0.1
Pan 82XP	Pancreas 2	0	0
Pro 12B	Prostate 1	0.3	0
Pro 23B	Prostate 2	3	4
Pro 13XB	Prostate 3	2	7
Pro 34B	Prostate 4	0.54	4.01
Pro 20XB	Prostate 5	4.8	4.3
Pro 65XB	Prostate 6	0.7	1.3
Tst 39X	Testis 1	2.78	0
End 8XA	Endometrium 1	0	0.2
Utr 85XU	Uterus 1	1.26	0

0= Negative

When matching samples were analyzed, the higher levels of expression were in the colon, showing a high degree of tissue specificity for this tissue. These results confirm the tissue specificity results obtained with the panel of normal pooled samples (Table 2). Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 3 shows overexpression of Cln106 (SEQ ID NO:3) in 15 colon cancer tissues compared with their respective normal adjacent (colon samples #1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 13, 16, 20, 21, and 22). There is overexpression in the cancer tissue for 65% of the colon matching samples tested (total of 23 colon matching samples). The matching sample Pan 71XL is a secondary cancer in pancreas, the primary cancer in that individual was a duodenal cancer.

Altogether, the high level of tissue specificity, plus the mRNA overexpression in 65% of the colon matching samples

tested are demonstrative of CSG Cln106 (SEQ ID NO:3) being a diagnostic marker for colon cancer.

Measurement of SEQ ID NO:4; Clone ID 816257; Gene ID 406452 (Cln107)

Absolute numbers as depicted in Table 4 are relative levels of expression of CSG Cln107 (SEQ ID NO:4) in 12 normal different tissues. All the values are compared to normal small intestine (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 4: Relative levels of Cln107 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	3.2
Endometrium	0
Kidney	0.2
Liver	0
Ovary	0
Pancreas	0
Prostate	0.1
Small Intestine	1
Spleen	0
Stomach	0.3
Testis	0
Uterus	0

The relative levels of expression in Table 4 show that mRNA expression of the CSG Cln107 (SEQ ID NO:4) is more than 10 fold higher in the pool of normal ascending colon (3.2), five fold higher in small intestine (1), and 1.5 fold higher in stomach (0.3), compared with the next higher expressor (0.2 for kidney). Seven of the pooled tissues samples analyzed were negative and prostate showed a relative expression of 0.1 for Cln107 (SEQ ID NO:4). These results demonstrate that Cln107 mRNA expression is highly specific for colon, small intestine, and in a lower degree for stomach.

The absolute numbers in Table 4 were obtained analyzing pools of samples of a particular tissue from different

individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 5.

The absolute numbers in Table 5 are relative levels of expression of Cln107 (SEQ ID NO:4) in 57 pairs of matching samples. All the values are compared to normal small intestine (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 5: Relative levels of Cln107 Expression in Individual Samples

Sample ID	Tissue	Cancer	Matching Normal Adjacent
Sto AC93	Stomach 1	8.9	13.4
Sto AC99	Stomach 2	6.0	0.9
Sml 21XA	Small Intestine 1	1.07	1.42
Sml H89	Small Intestine 2	0.97	4.13
Cln B56	Colon-Cecum(A)1	2	16
Cln AS45	Colon-Ascending(A)2	0.7	2.1
Cln CM67	Colon-Cecum(B)3	1.6	2.1
Cln AS67	Colon-Ascending(B)4	1.2	6.2
Cln AS43	Colon-Ascending(C)5	13.5	0.5
Cln AS46	Colon-Ascending(C)6	9.7	23.6
Cln AS98	Colon-Ascending(C)7	28.1	1.4
Cln AS89	Colon-Ascending(D)8	0.9	3.1
Cln TX01	Colon-Transverse(B)9	3.0	10.6
Cln TX89	Colon-Transverse(B)10	4.5	0.6
Cln TX67	Colon-Transverse(C)11	3.6	3.4
Cln MT38	Colon-Splenic Flexture(M)12	4.0	2.6
Cln SG89	Colon-Sigmoid(B)13	4.7	0.9

Cln SG67	Colon-Sigmoid(C)14	1.0	1.3
Cln SG33	Colon-Sigmoid(C)15	14.2	7.6
Cln SG45	Colon-Sigmoid(D)16	4.8	6.0
Cln B34	Colon-Rectosigmoid(A)17	3	2
Cln CXGA	Colon-Rectum(A)18	4.4	1.9
Cln RC67	Colon-Rectum(B)19	0.1	0.4
Cln C9XR	Colon-Rectosigmoid(C)20	5	3
Cln RS45	Colon-Rectosigmoid(C)21	11.4	4.6
Cln RC01	Colon-Rectum(C)22	1.8	2.3
Cln RC89	Colon-Rectum(D)23	0.1	5.35
Bld 46XK	Bladder 1	0.2	0
Bld 66X	Bladder 2	1	1
Bld 32XK	Bladder 3	0.1	0.1
Kid 126XD	Kidney 1	0	0.02
Kid 12XD	Kidney 2	0.1	0.2
Kid 5XD	Kidney 3	0.3	0.0
Kid 6XD	Kidney 4	0.1	0.1
Kid 106XD	Kidney 5	0.0	0.1
Liv 42X	Liver 1	7.9	0.002
Liv 15XA	Liver 2	0.0	0.0
Liv 94XA	Liver 3	0.0	0.0
Lng AC69	Lung 1	1.6	0.2
Lng BR94	Lung 2	0.4	0
Lng 47XQ	Lung 3	0.78	0.2
Mam 59X	Mammary Gland 1	0.05	0.3
Mam B011X	Mammary Gland 2	0.01	0.004
Mam A06X	Mammary Gland 3	0.22	0
Ovr 103X	Ovary 1	0.01	0.01
Ovr 130X	Ovary 2	0.09	0.1
Pan 71XL	Pancreas 1	2.51	2.81

Pan 82XP	Pancreas 2	0	0.62
Pro 12B	Prostate 1	0.3	0.1
Pro 23B	Prostate 2	0.3	0.2
Pro 13XB	Prostate 3	0	0
Pro 34B	Prostate 4	0.04	0.22
Pro 20XB	Prostate 5	0.4	0.1
Pro 65XB	Prostate 6	0.0	0.1
Tst 39X	Testis 1	0.02	0.01
End 8XA	Endometrium 1	0.01	0.5
Utr 85XU	Uterus 1	0.03	0

0= Negative

When matching samples were analyzed, the higher levels of expression were in colon, stomach, and small intestine, showing a high degree of tissue specificity for colon tissues. These results confirm the tissue specificity results obtained with normal pooled samples (Table 4). Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 5 shows overexpression of Cln107 (SEQ ID NO:4) in 11 colon cancer tissues compared with their respective normal adjacent (colon samples #5, 7, 10, 11, 12, 13, 15, 17, 18, 20, and 21). There is overexpression in the cancer tissue for 48% of the colon matching samples tested (total of 23 colon matching samples). The matching sample Pan 71XL is a secondary cancer in pancreas, the primary cancer in that individual was a duodenal cancer.

Altogether, the high level of tissue specificity, plus the mRNA overexpression in almost half of the colon, stomach, and small intestine matching samples tested are demonstrative

of CSG Cln107 (SEQ ID NO:4) being a diagnostic marker for colon cancer.

Measurement of SEQ ID NO:5; Clone ID 775133; Gene ID 24508 (Cln108)

The absolute numbers shown in Table 6 are relative levels of expression of CSG Cln108 (SEQ ID NO:5) in 12 normal different tissues. All the values are compared to normal small intestine (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 6: Relative levels of Cln108 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	2846.5
Endometrium	1
Kidney	5.5
Liver	18.7
Ovary	3.4
Pancreas	198.1
Prostate	1024
Small Intestine	810.8
Spleen	32.2
Stomach	9981.2
Testis	0
Uterus	294.1

The relative levels of expression in Table 6 show that mRNA expression of CSG Cln108 (SEQ ID NO:5) is more than 10 fold higher in the pool of normal ascending colon (2846.5) and almost ten fold higher in stomach (9981.2), compared to the expression level in any other tissue analyzed. These results demonstrate that mRNA expression of this CSG is also highly specific for colon and stomach.

The absolute numbers in Table 6 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 7.

The absolute numbers depicted in Table 7 are relative levels of expression of Cln108 (SEQ ID NO:5) in 57 pairs of matching samples. All the values are compared to normal small intestine (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 7: Relative levels of Cln108 Expression in Individual Samples

Sample ID	Tissue	Cancer	Matching Normal Adjacent
Sto AC93	Stomach 1	28696	34842
Sto AC99	Stomach 2	21523	30862
Sml 21XA	Small Intestine 1	2944	964.4
Sml H89	Small Intestine 2	244.5	3513.2
Cln B56	Colon-Cecum(A)1	27242	24637
Cln AS45	Colon-Ascending(A)2	5827.0	8771.0
Cln CM67	Colon-Cecum(B)3	4251.0	4684.0
Cln AS67	Colon-Ascending(B)4	564.0	1949.0
Cln AS43	Colon-Ascending(C)5	50310	10949
Cln AS46	Colon-Ascending(C)6	246044	120073
Cln AS98	Colon-Ascending(C)7	40442	17482
Cln AS89	Colon-Ascending(D)8	5730.0	1581.0
Cln TX01	Colon-Transverse(B)9	22281.0	114784.0
Cln TX89	Colon-Transverse(B)10	11026.0	1639.0
Cln TX67	Colon-Transverse(C)11	17004.0	11654.0
Cln MT38	Colon-Splenic Flexture(M)12	77589	31620
Cln SG89	Colon-Sigmoid(B)13	140339.0	49617.0
Cln SG67	Colon-Sigmoid(C)14	4951.0	7905.0
Cln SG33	Colon-Sigmoid(C)15	60875	120490

Cln SG45	Colon-Sigmoid(D)16	30437.0	47267.0
Cln B34	Colon-Rectosigmoid(A)17	5848	5861
Cln CXGA	Colon-Rectum(A)18	13877.0	9787.0
Cln RC67	Colon-Rectum(B)19	1703.0	26589.0
Cln C9XR	Colon-Rectosigmoid(C)20	2458	19071
Cln RS45	Colon-Rectosigmoid(C)21	95523	61939
Cln RC01	Colon-Rectum(C)22	98891.0	80047.0
Cln RC89	Colon-Rectum(D)23	17.0	1775
Bld 46XK	Bladder 1	0	8
Bld 66X	Bladder 2	397	44
Bld 32XK	Bladder 3	0.0	16.0
Kid 126XD	Kidney 1	32	22
Kid 12XD	Kidney 2	6	0
Kid 106XD	Kidney 3	4.0	33.0
Liv 42X	Liver 1	4783	0
Liv 15XA	Liver 2	4.0	10.0
Liv 94XA	Liver 3	159.0	21.0
Lng AC69	Lung 1	222	295
Lng BR94	Lung 2	112	0
Lng 47XQ	Lung 3	30	69
Lng AC66	Lung 4	29	137
Mam 59X	Mammary Gland 1	56	0
Mam B011X	Mammary Gland 2	54	31
Mam A06X	Mammary Gland 3	12	0
Ovr 103X	Ovary 1	37	0
Pan 71XL	Pancreas 1	13203	4163
Pan 82XP	Pancreas 2	39.1	0
Pro 12B	Prostate 1	386	88
Pro 23B	Prostate 2	250	23
Pro 13XB	Prostate 3	92	731

Pro 34B	Prostate 4	33.3	265.7
Pro 20XB	Prostate 5	454.6	1908.9
Pro 65XB	Prostate 6	733.5	922.0
End 8XA	Endometrium 1	5	92
Utr 85XU	Uterus 1	98.9	21.8
Utr 23XU	Uterus 2	35.3	0
Utr 135XO	Uterus 3	39.2	43.8
Utr 141XO	Uterus 4	212.1	55.9

0= Negative

When matching samples were analyzed, the higher levels of expression were in colon and stomach, showing a high degree of tissue specificity for these two tissues. These results confirm the tissue specificity results obtained with normal pooled samples (Table 6). Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 7 shows overexpression of CSG Cln108 (SEQ ID NO:5) in 13 colon cancer tissues compared with their respective normal adjacent (colon samples #1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 18, 21, and 22). There is overexpression in the cancer tissue for 56% of the colon matching samples tested (total of 23 colon matching samples). The matching sample Pan 71XL is a secondary cancer in pancreas, the primary cancer in that individual was a duodenal cancer.

Altogether, the high level of tissue specificity, plus the mRNA overexpression in more than half of the colon, stomach, and small intestine matching samples tested are demonstrative of this CSG, Cln108 (SEQ ID NO:5), also being a diagnostic marker for colon cancer.

Measurement of SEQ ID NO:7; Clone ID 2348122; Gene ID 23371 (Cln109)

The absolute numbers depicted in Table 8 are relative levels of expression of CSG Cln109 (SEQ ID NO:7) in 12 normal different tissues. All the values are compared to normal ovary (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 8: Relative levels of Cln109 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	28.8
Endometrium	0.45
Kidney	0.41
Liver	0.72
Ovary	0.07
Pancreas	82.8
Prostate	124.3
Small Intestine	626.4
Spleen	1.2
Stomach	12.05
Testis	1.51
Uterus	52.99

The relative levels of expression in Table 8 show that mRNA expression of CSG Cln109 (SEQ ID NO:7), is more than 5 fold higher in the pool of normal small intestine (626.4) compared to the expression level in any other tissue analyzed. These results demonstrate that Cln109 (SEQ ID NO:7) mRNA expression is highly specific for small intestine

The absolute numbers in Table 8 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 9.

The absolute numbers depicted in Table 9 are relative levels of expression of Cln109 (SEQ ID NO:7) in 53 pairs of matching samples. All the values are compared to normal ovary (calibrator). A matching pair is formed by mRNA from the

cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 9: Relative levels of Cln109 Expression in Individual Samples

Sample ID	Tissue	Cancer	Matching Normal Adjacent
Sto AC93	Stomach 1	2574	1310
Sto AC99	Stomach 2	4153	5
Sml 21XA	Small Intestine 1	2667	13663.8
Sml H89	Small Intestine 2	57.8	904.29
Cln B56	Colon-Cecum(A)1	6794	299
Cln AS45	Colon-Ascending(A)2	814.6	105.8
Cln CM67	Colon-Cecum(B)3	294.6	36.1
Cln AS67	Colon-Ascending(B)4	2.2	26.3
Cln AS43	Colon-Ascending(C)5	111	377
Cln AS46	Colon-Ascending(C)6	1180	352
Cln AS98	Colon-Ascending(C)7	1075	92
Cln AS89	Colon-Ascending(D)8	14022.7	87.5
Cln TX01	Colon-Transverse(B)9	1027.6	282.1
Cln TX89	Colon-Transverse(B)10	2.5	23.7
Cln TX67	Colon-Transverse(C)11	0.1	72.3
Cln MT38	Colon-Splenic Flexture(M)12	372	88
Cln SG89	Colon-Sigmoid(B)13	179.2	33.4
Cln SG67	Colon-Sigmoid(C)14	85.0	94.7
Cln SG33	Colon-Sigmoid(C)15	5461	377
Cln SG45	Colon-Sigmoid(D)16	762.7	15.9
Cln B34	Colon-Rectosigmoid(A)17	460	1
Cln RC67	Colon-Rectum(B)18	64.5	136.2
Cln C9XR	Colon-Rectosigmoid(C)19	441	34
Cln RS45	Colon-Rectosigmoid(C)20	1931	195

Cln RC01	Colon-Rectum(C)21	72.8	19.1
Cln RC89	Colon-Rectum(D)22	4.8	90.2
Bld 46XK	Bladder 1	4	3
Bld 66X	Bladder 2	1	0
Bld 32XK	Bladder 3	0.1	307.6
Kid 126XD	Kidney 1	0	2
Kid 12XD	Kidney 2	3	16
Kid 5XD	Kidney 3	0.0	0.3
Kid 6XD	Kidney 4	18.5	1.2
Liv 42X	Liver 1	21	0.03
Liv 15XA	Liver 2	0.5	0.4
Liv 94XA	Liver 3	0.4	0.0
Lng AC69	Lung 1	0.1	0
Lng BR94	Lung 2	3	0
Lng 60XL	Lung 3	0.1	0
Mam 59X	Mammary Gland 1	0	4
Mam B011X	Mammary Gland 2	8	13
Mam A06X	Mammary Gland 3	4.7	9.6
Pan 71XL	Pancreas 1	8902.5	1428.2
Pan 82XP	Pancreas 2	0.2	9.3
Pro 12B	Prostate 1	9	20
Pro 23B	Prostate 2	191	88
Pro 13XB	Prostate 3	12	460
Pro 34B	Prostate 4	3.2	80.4
Tst 39X	Testis 1	29.9	0
End 8XA	Endometrium 1	0.3	21
Utr 85XU	Uterus 1	244.7	592.2
Ovr 63A	Ovary 1	11.4	0
Ovr A1C	Ovary 2	68.4	0

0= Negative

When matching samples were analyzed, the higher levels of expression were in small intestine, colon and stomach, showing a high degree of tissue specificity for these three colon tissues. These results confirm the tissue specificity results obtained with normal pooled samples for small intestine (Table 8). Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 9 shows overexpression of CSG, Cln109 (SEQ ID NO:7) in 15 colon cancer tissues compared with their respective normal adjacent (colon samples #1, 2, 3, 6, 7, 8, 9, 12, 13, 15, 16, 17, 19, 20, and 21). There is overexpression in the cancer tissue for 68% of the colon matching samples tested (total of 22 colon matching samples). The matching sample Pan 71XL is a secondary cancer in pancreas, the primary cancer in that individual was a duodenal cancer.

Altogether, the high level of tissue specificity, plus the mRNA overexpression in more than half of the colon, stomach, and small intestine matching samples tested are demonstrative of CSG Cln109 (SEQ ID NO:7) being a diagnostic marker for colon cancer. The amino acid sequence encoded by the open reading frame of Cln109 is depicted in SEQ ID NO:10.

What is claimed is:

1. A method for diagnosing the presence of colon cancer in a patient comprising:

(a) measuring levels of CSG in a sample of cells, tissue or bodily fluid obtained from the patient; and

(b) comparing the measured levels of CSG with levels of CSG in a sample of cells, tissue or bodily fluid obtained from a control, wherein an increase in measured levels of CSG in the patient versus the CSG levels in the control is associated with the presence of colon cancer.

2. A method of diagnosing metastatic colon cancer in a patient comprising:

(a) measuring levels of CSG in a sample of cells, tissue, or bodily fluid obtained from the patient; and

(b) comparing the measured levels of CSG with levels of CSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in measured CSG levels in the patient versus the CSG levels in the control is associated with a cancer which has metastasized.

3. A method of staging colon cancer in a patient comprising:

(a) identifying a patient suffering from colon cancer;

(b) measuring levels of CSG in a sample of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the measured levels of CSG with levels of CSG in a sample of cells, tissue, or bodily fluid obtained from a control, wherein an increase in the measured levels of CSG versus the levels of CSG in the control is associated with a cancer which is progressing and a decrease in the measured levels of CSG versus the levels of CSG in the control is associated with a cancer which is regressing or in remission.

4. A method of monitoring colon cancer in a patient for the onset of metastasis comprising:

(a) identifying a patient having colon cancer that is not known to have metastasized;

(b) periodically measuring CSG levels in samples of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the periodically measured levels of CSG with levels of CSG in cells, tissue, or bodily fluid obtained from a control, wherein an increase in any one of the periodically measured levels of CSG in the patient versus the levels of CSG in the control is associated with a cancer which has metastasized.

5. A method of monitoring changes in a stage of colon cancer in a patient comprising:

(a) identifying a patient having colon cancer;

(b) periodically measuring levels of CSG in samples of cells, tissue, or bodily fluid obtained from the patient; and

(c) comparing the measured levels of CSG with levels of CSG in a sample of the same cells, tissue, or bodily fluid of a control, wherein an increase in any one of the periodically measured levels of CSG versus levels of CSG in the control is associated with a cancer which is progressing in stage and a decrease in any one of the periodically measured levels of CSG versus the levels of CSG in the control is associated with a cancer which is regressing in stage or in remission.

6. The method of claim 1, 2, 3, 4 or 5 wherein the CSG comprises SEQ ID NO:3, 4, 5 or 7.

SEQUENCE LISTING

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10498

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; G01N 33/53, 33/574
US CL :435/4, 6, 7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 6, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Scisearch, HCAPLUS, medline, biosis, membase, wpids, jicst-eplus, biobusiness, biotechds, phin, phic, embal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/39419 A1 (HUMAN GENOME SCIENCES, INC.) 12 December 1996, see entire document.	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search: 16 AUGUST 1999

Date of mailing of the international search report: 10 SEP 1999

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D. Lawrence for
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10498

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 6
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The sequence listing in computer readable form did not comply with required standards. The disc could not be preocessed. Thus, a search of claim 6, drawn to SEQ ID NO:3, 4, 5 or 7 , could not be carried out.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.